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Effects of fish-farm biodeposition on periphyton assemblages on artificial substrates in the southern Tyrrhenian Sea (Gulf of Castellammare, Sicily)

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Abstract An algal assemblage growing on artificial substrata of fish-farm cages was investigated. Specifically, algal response to the effects of fish-farm facilities was studied, in order to identify a possible future descriptor of biodeposition impact. Some sites were positioned upstream of the farms (at least 750 m; 'controls') and other sites were positioned downstream of the farms ('impacts'). All sites were situated in the Tyrrhenian Sea. Control and impact sites differed significantly with regard to the dissolved nutrient profile. The fouling community (samples were scraped from buoys) displayed a reduction gradient in diversity which increased with the effect of fish farms. A total of 51 taxa were identified (three Cyanophyceae, three Phaeophyceae, five Bacillariophyceae, three Chlorophyceae, six Ulvophyceae and 31 Rhodophyceae), with a dominance of opportunistic species (with *r* strategy). A general increase in values of the Rhodophyceae by Phaeophyceae ratio (*R*/*P*) were recorded, indicating a remarkable impact of nutrient enrichment from fish culture facilities on an algal community structure.

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Introduction

Aquaculture in the Mediterranean, mainly developed in coastal environments, has been expanding at a remarkable rate over the last decade, raising serious concerns about the impact of fish-farm biodeposition (Sarà et al. 2006). Fish-farm nutrient enrichment (i.e. incomplete consumption of food by farmed fish and the excretion of nitrogen compounds; Beveridge 1996) affect the environment by modifying the physical and chemical characteristics in the surrounding area (Holmer 1991; Sarà 2007). Such modifications can have a remarkable effect both on the water column and the sediment chemistry (Holmer 1991; Iwama 1991; Wu et al. 1994; Pearson and Black 2000; Kalantzi and Karakassis 2006; Sarà 2007). Consequently, chemical changes in the environment can also elicit deviations from natural common patterns of benthic assemblage response. Indeed, under eutrophication, benthos can selectively respond, reducing its richness, diversity levels and/or enhancing its biomass per surface unit (Mirto et al. 2002). Although the response of almost all organisms to effects of fish-farm biodeposition has been investigated in the current literature (e.g. Mirto et al. 2002) to date, little is known about the impact on the macroalgal communities of artificial substrata.

However, such colonisation (defined as periphyton or algal fouling; Mook 1981) by local infra-upper-littoral benthic communities can provide reliable records of pollution effects (Angel and Spanier 2002). In farms the world over, there is a wide availability of artificial hard surfaces, e.g. buoys and cage structures, all functioning as substrata for the recruitment of many intertidal benthic organisms. Recently, several studies have pointed out that fouling species can be reliably used as indicators of environmental disturbance (Calcagno et al. 1998) both from organic (Kalaman 2001, Mayer-Pinto and Junqueira 2003; Sarà et al. 2007) and thermal pollution (Zvyagintsev and Korn 2003). However, very little information has been published about farming waste impact on periphyton growing on artificial substrata (Díaz Villanueva et al. 2000) around cages and its role as a bioindicator. Indeed, the data available in current literature generally show the effects of aquaculture pollution on benthic coastal macroalgae (Ruokolahti 1988; Rönnberg et al. 1992). However, the phytobenthic community, due to its ability to integrate biotic and abiotic factors over time, can directly respond to the changes in abiotic variables, resulting in sensitive bioindicators of ecosystem changes at smaller spatial scales.

Numerous indices can be employed in assessing environmental characteristics on the basis of marine vegetation. Amongst others, the Rhodophyceae by Phaeophyceae ratio (*R*/*P*), Environmental Quality Value index (EQV; Torras et al. 2003) and Ecological Evaluation Index (EEI; Orfanidis et al. 2003) have been used. In order to test the efficiency of periphyton as a bioindicator of organic pollution, the present study aimed to investigate the effects of fish-farm biodeposition on a macroalgal assemblage by comparing in controls and impacts (1) its richness, diversity and community structure, and (2) its Rhodophyceae by Phaeophyceae ratio (*R*/*P* index).

Materials and methods

Study area and sample collection

The study was carried out in 2004 in the Gulf of Castellammare (Southern Tyrrhenian Sea, LAT 38°02′31″ N; LONG 12°55′28″ E). The fish farm (Ittica Trappeto Inc.), composed of six floating and three submersible circular cages (Farmocean, Norway), is located about 2 km off the coast in the eastern part of the Gulf. The hydrodynamic regime of the area is characterised by a dominant current with an average speed of 12-20 cm s^{-1} which flows mono-directionally in a west–east direction (Sarà et al. 2006). The above-mentioned hydrodynamic conditions and absence of any other source of pollution from the study area (Sarà et al 2007) allowed us to assume that sites positioned upstream of the farms (at least 750 m) could be considered as controls (i.e. not affected by organic enrichment coming from farms), while sites positioned downstream from the farms could be considered as impacts (i.e. affected only by farm biodeposition with no other influences present). To test this hypothesis (i.e. differences among controls and impacts), sub-surface water samples were collected using Niskins and analysed for dissolved nutrients (nitrogen and phosphorus). Analyses were carried out according to Strickland and Parson (1972). Fouling samples were collected only from artificial substrata of buoys marking out the farming area, which had already been in the water for about 3 years before the sampling. No cleaning operations have ever been carried out on the buoys (Sarà et al. 2007), thus, sampling sites were represented by groups of buoys positioned both up- [CTRL] and downstream [IMP]. All buoys were of the same size (about 1.4 in diameter; the submerged sampling area was estimated to be not less than 1 m^2), shape and material (plastic), and the samples were randomly collected from substrata positioned immediately below the floatation line. The buoys were grouped in order to randomly select three plots of buoys. In each plot, we had at least three buoys and from each of them, one sample was taken from a quadrate (20 cm × 20 cm, 400 cm^2 surface area). Two sampling campaigns were carried out, in spring and autumn 2004, but only results of the latter were reported, as the low abundance of vegetal fouling in spring samples did not lend itself to analysis. Algae were scraped from artificial substrata on each quadrate with a putty knife, brought back to the laboratory and stored in a fridge (~4°C) for a few hours. Macroalgae were then separated from animals and fixed with 4% buffered formaldehyde in seawater solution and identified to genus or species level.

Statistical analyses and ecological indexes

Before investigating possible statistical differences among sites in algal assemblages, we tested differences among control and impact sites in nutrient concentrations using a three-way analysis of variance (ANOVA; Underwood 1997). Condition (upstream and downstream, 2 levels) was chosen as fixed factor, and three sites were treated at random and nested in Condition. Sampling months (October and November) were chosen as random factors. Three replicates were collected for each of the following variables: ammonium, nitrites, nitrates and orthophosphates. When a significant difference ($P < 0.05$) for the main effect was observed, the appropriate means were compared using Student-Newman-Keuls (SNK) tests (Underwood 1997). Data was transformed only when necessary, to meet the assumptions of the parametric statistics. Multivariate analyses were used to compare algal assemblages among control and impact sites (a procedure commonly used to test multivariate differences among different factorial conditions; but see Benedetti-Cecchi et al. 2001). A matrix of similarities between each pair of samples was calculated using the Bray-Curtis similarity coefficient (Bray and Curtis 1957) on untransformed data, and a one-way ANOSIM (analysis of similarities, a permutation-based hypotheses testing procedure, analogous to univariate ANOVA; Clarke 1993) was performed to test for similarity differences among sites. Furthermore, a similarity percentage (SIMPER) procedure was carried out to weight the percentage contribution of each taxon to the pairwise differences among controls and impacts (Clarke 1993). Non-metric multidimensional scaling (nMDS) was used to produce two-dimensional ordinations of the rank orders of similarities in the different conditions (Underwood and Anderson 1994, Anderson and Underwood 1997). The R/P index and the mean number of species for each sample were also calculated to give an indication of the state of health of the study area. GMAV (University of Sydney, Australia; personally licensed to G. Sarà) and the PRIMER package (Plymouth Marine Laboratory, UK) were used to perform uni-and multivariate analyses.

Results

Ammonium and nitrate concentrations were higher in impact than in control sites, which constituted a significant difference (ANOVA, $P < 0.05$). Nitrites and orthophosphate were similar in both conditions (Fig. 1). Algal community grew exclusively as epibiont (on algae, mussels or hydroid) and covered in general about 25–30% of living substrates. A total of 51 algal taxa were classified (three Cyanophyceae, three Phaeophyceae, five Bacillariophyceae, three Chlorophyceae, six Ulvophyceae and 31 Rhodophyceae). The most frequent genera were *Ceramium, Polysiphonia* and *Cladophora*. Species richness increased moving from IMP 1 (31) and IMP 2 (30) to IMP 3 (36), with IMP 3 not showing significant differences in respect to controls (37). In the control sites, periphyton showed total coverage values significantly lower ($P < 0.05$) than those recorded in the impacts. Farm effluents induced an increasing effect on algal coverage and a decreasing effect on species richness. The *R*/*P* index displayed significantly high values in all sites (>6), due to the dominance of the Rhodophyceae and the low number of Phaeophyceae. In particular, *R*/*P* values decreased moving from IMP 1 to IMP 3 and the control sites. The epiphytic community were particularly abundant, with a high percentage of diatoms; among them the most represented genera were *Navicula* and *Licmophora*. A dominance of uni-seriate or thin corticated filamentous thalli (e.g. *Cladophora* and *Polysiphonia*) and foliose algae (e.g. *Enteromorpha*) was found. The dominance of annual species, characterised by a high

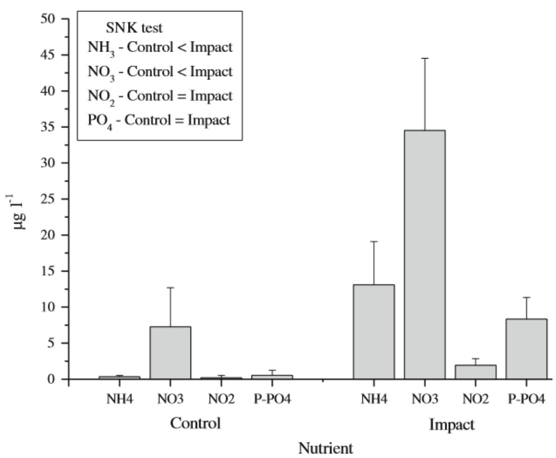

Fig. 1 Nutrient differences among CTRL (controls) and IMP (impact) sites and the outcome of ANOVA (Student-Newman-Keuls [SNK] test was reported inside the graph; NH_4 = ammonia; NO_2 = nitrites; NO_3 = nitrates; P-PO_4 = orthophosphates)

rate of growth and a short life cycle (opportunistic, with *r* strategy), corresponded to ESG II (90%). MDS ordination (Fig. 2a), based on percentage of coverage and number of species, and the cluster analysis (Fig. 2b) highlighted a separation of the four sites and a gradient from IMP 1 to the control sites, while a high similarity between IMP 3 and the control sites was displayed. In ANOSIM, the *R*-value is scaled to lie between −1 and + 1, the value of zero representing no difference between the set of samples. In accordance with Clarke and Gooley (2001) *R*-values > 0.75 were interpreted as well-separated. ANOSIM (Table 1) showed that controls were significantly different from the impacts, although negligible with IMP 3. Different species contributed to the dissimilarity among sites (Table 2). In particular, the same number of taxa (respectively, eight and seven) contributed to differences among controls and IMP 1 and 2 (the maximum and the intermediate impacts observed), while only four taxa made the majority contribution to differences between IMP 3 and controls (the minimum level).

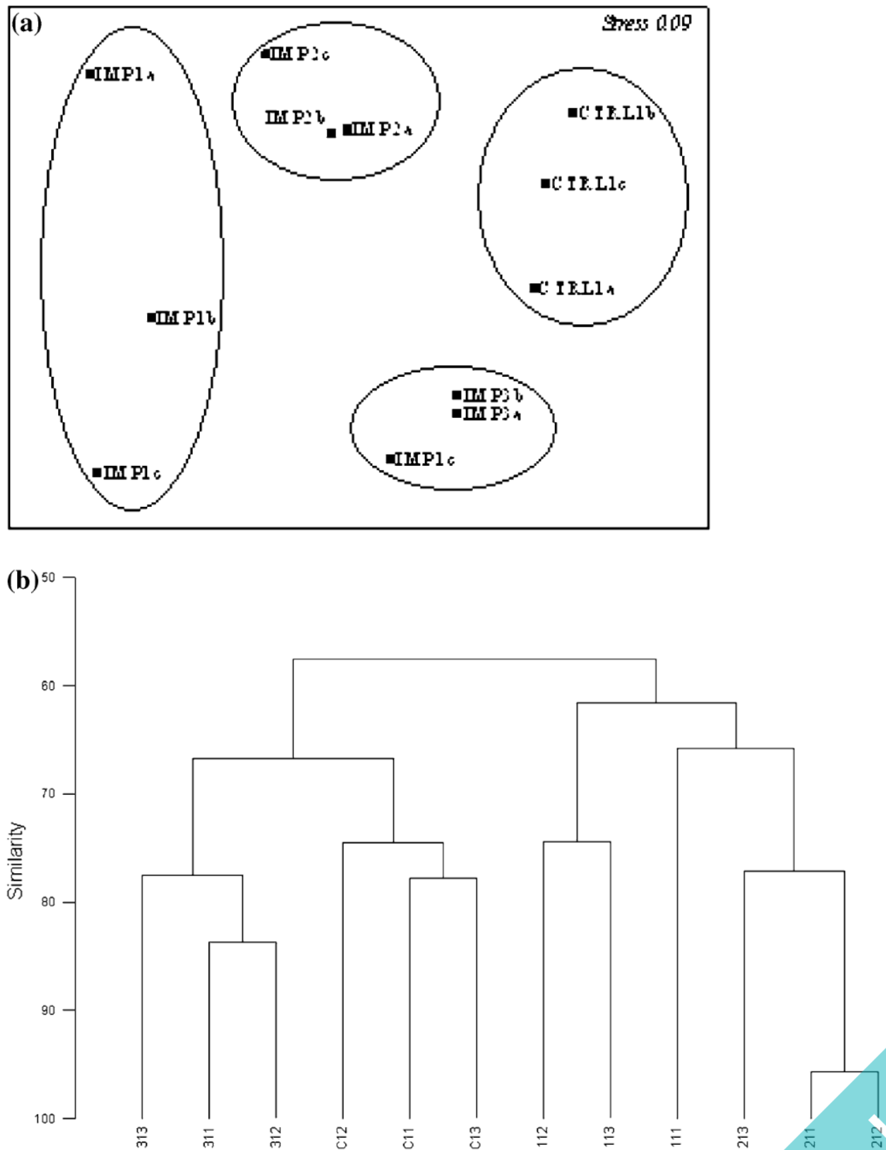

Fig. 2 Multidimensional scaling (**a**) and cluster analysis (**b**) carried out on the Bray-Curtis similarity matrix to test the differences among controls and putative impacts

Table 1 *R* values from ANOSIM comparing controls and impacts (CTRL = control; IMP = impact; *P* = probability level)

Pairwise test	*R*	*P*
CTRL vs. IMP 1	1.0	< 0.01
CTRL vs. IMP 2	1.0	< 0.01
CTRL vs. IMP 3	0.8	< 0.01
IMP 1 vs. IMP 2	0.7	< 0.01
IMP 1 vs. IMP 3	0.9	< 0.01
IMP 2 vs. IMP 3	1.0	< 0.01
Global R	0.9	=0.1

Discussion

Fish-farm biodeposition may cause physical and chemical changes in the area surrounding farms by modifying characteristics of the mediolittoral benthic environments. Similar biological responses in other types of organisms influenced by fish farms have been observed (Hargrave et al. 1997; Pearson and Black 2000; La Rosa et al. 2001; Mirto et al. 2002; Sarà et al. 2006; Sarà et al. 2007), but rarely has an effect been demonstrated on periphyton attached to artificial substrata (Díaz Villanueva et al. 2000). In the present study area, the response of the algal community colonising artificial substrata and affected by fish-farm biodeposition was significant and most probably linked to changes in water chemistry. Changes were displayed in composition and species richness, showing an optimal degree of sensitivity to the fish-farm disturbance. Such a response was particularly evident when we looked at diversity and species richness, which were significantly lower under high degrees of nutrient enrichment (downstream/impact sites) than in controls (upstream). Fish-farm influence elicited a selection of opportunistic species (annual, *r* strategy, belonging to the ESG II group), which had high net productivity, rapid growth, high output of reproductive bodies and the tendency to be more eurytopic. This was in agreement with other studies that have shown high ephemerality at polluted sites, when stimulated by nutrient enrichment (Diaz et al. 2002). In particular, assemblages at IMP 1 and 2, closer to the direct emission from fish farms, were markedly different compared to controls. The greater the effect of organic enrichment (IMP 1 and 2), the lower the number of taxa. Accordingly, the percentage of algal coverage also varied, as values were higher in impacted sites than in controls. This suggests that coverage was directly correlated with farm nutrient enrichment, while species richness was inversely correlated. Under high coverage situations, as recorded in impacted sites, foliose algae (e.g. *Enteromorpha*) dominated the assemblage as a response to the nutrient enrichment. Most importantly, the *R*/*P* index indicated clearly the impact of nutrient enrichment. Indeed, *R*/*P* values were higher than six, suggesting a presence of overall disturbance but with subtle differences among sites. High *R*/*P* values observed in impacts were basically due to the low abundance of Phaeophyceae (i.e. negatively affected by environmental disturbance). In control sites, Phaeophyceae being higher in number, the *R*/*P* index reached lower values. Such a picture, describing a different effect of farm effluents on vegetal fouling, could be confirmed by the outcome of multivariate analyses. Indeed, a significant gradient of dissimilarity between IMPs 1, 2 and 3 and control values was quite evident, suggesting that the higher the effects (as in IMP 1), the higher the dominance of genera *Enteromorpha* and *Cladophora*.

Conclusions

Our study shows that descriptors of algal communities on artificial substrata, like the *R*/*P* index, number

Table 2 List of taxa mostly contributing to the dissimilarity among control and impact sites (only top species are reported)

Sites	Species
CTRL vs. IMP 1	*Enteromorpha intestinalis* v. *intestinalis, Cladophora dalmatica, Sphacelaria* sp, *Ulvella lens, Spyridia filamentosa, Herposiphonia secunda, Erythrotrichia carnea, Erythrocladia irregularis*
CTRL vs. IMP 2	*Ceramium siliquosum* v. siliquosum, *Ceramium deslongchampsii, Ectocarpus siliculosus* v. *siliculosus, Spyridia filamentosa, Lophosiphonia obscura, Erythrotrichia carnea, Erythrocladia irregularis*
CTRL vs. IMP 3	*Erythrotrichia carnea, Titanoderma pustulatum, Heterosiphonia crispella, Ceramium siliquosum* v. *siliquosum*

of species and its composition, give a good indication of the effect of inorganic biodeposition from fish farms. We found that algal assemblages on artificial substrata are sensitive to nutrient enrichment, adopting different strategies: (1) a decrease in species richness with a selection of opportunistic species (with *r* strategy), (2) an increase in coverage of green algae, and (3) selecting paucispecific populations characterised by more specialised organisms. Thus, although periphyton has been rarely chosen as a descriptor of fish farming impact, results from the present study advocates its use in environmental assessment protocols. However, before considering it as reliable descriptor of biodeposition impact, further manipulative studies are needed to better understand its response to different substrata types and possible seasonal changes of the community structure.

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